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REPORT DOCUMENTATION PAGE				Form Approved OMB No 0704-0188	
1a REPORT SECURITY CLASSIFICATION (U)			1b RESTRICTIVE MARKINGS NA		
AD-A212 667			3 DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited		
			5 MONITORING ORGANIZATION REPORT NUMBER NA		
6a NAME OF PERFORMING ORGANIZATION Southern Illinois University at Carbondale		6b OFFICE SYMBOL (If applicable) NA	7a NAME OF MONITORING ORGANIZATION Office of Naval Research		
6c ADDRESS (City, State, and ZIP Code) Dept. of Chemistry & Biochemistry Carbondale, Illinois 62901-4409			7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		
8a NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research		8b OFFICE SYMBOL (If applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-K-0739		
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000			10 SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO 61153N	PROJECT NO RR04106	TASK NO 441d-010
			WORK UNIT ACCESSION NO		
11 TITLE (Include Security Classification) (U) Structure and expression of various RNAs in the Archaeobacteria					
12 PERSONAL AUTHOR(S) Gupta, Ramesh					
13a TYPE OF REPORT Annual		13b TIME COVERED FROM 09/88 TO 08/89		14 DATE OF REPORT (Year, Month, Day) 89/09/05	
15 PAGE COUNT 2					
16 SUPPLEMENTARY NOTATION					
17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD 06	GROUP 03	SUB-GROUP	Archaeobacteria, Thermophiles, tRNA, Intron, Modified Nucleosides, tRNA-methyltransferases		
19 ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>A novel transfer RNA gene of <u>Sulfolobus</u> has been cloned. It has a large "D-loop" containing 27 residues and appears to code for glutamic acid tRNA. The sequence CCGGU occurs twice within the "D-loop". Two symmetrical cuts within or just outside these sequences can produce a typical tRNA and an 18-base "intron". The gene occurs as a single copy in the genome and is transcribed. It is being determined that whether this "intron" is actually spliced out or it remains as a part of the mature tRNA.</p> <p>In addition, an approximately 9 Kb <u>EcoRI</u> genomic fragment of <u>Sulfolobus</u>, containing genes for two tRNA-methyltransferases has been cloned. The tRNAs isolated from <u>E. coli</u> cells containing this recombinant plasmid contain N²-methylguanosine (m²G) and 1-methyladenosine (m¹A), the modified nucleosides not normally present in <u>E. coli</u> tRNAs. It appears that some <u>Sulfolobus</u> genes are expressed when cloned in <u>E. coli</u> and at least some of its enzymes are functional in the <u>E. coli</u> environment.</p>					
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21 ABSTRACT SECURITY CLASSIFICATION (U)		
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. M. Marron			22b TELEPHONE (Include Area Code) 202-696-4760		22c OFFICE SYMBOL ONR

DD Form 1473, JUN 86

Previous editions are obsolete

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S/N 0102-LF-014-6603

89 9 13 005

ANNUAL REPORT



CONTRACT NO.: N00014-86-K-0739
R&T Code: 4412010

FULL TITLE: Structure and expression of various RNAs in the Archaeobacteria.

ABBREVIATED TITLE: RNAs in Archaeobacteria

INSTITUTION: The Board of Trustees of Southern Illinois University,
Carbondale, IL 62901

PRINCIPAL INVESTIGATOR: Ramesh Gupta
Chemistry and Biochemistry
Southern Illinois University
Carbondale, IL 62901

PERIOD OF REPORT: September 1988 - August 1989

Summary of Project Goals:

1. Separation and sequencing of various RNAs of thermophilic archaeobacteria.
2. Sequencing of various tRNA genes of archaeobacteria (mainly thermophiles) and their surrounding regions to determine the organization of these genes and to identify the potential transcription control regions and the transcript processing sites.
3. Identification of the transcription initiation and termination sites in tRNA genes of various archaeobacteria.
4. Characterization of RNA processing in thermophilic archaeobacteria, initially using small transcripts, e.g., tRNA gene transcripts.

Recent Accomplishments

We have partially sequenced specific regions of some of the previously produced clones containing Sulfolobus tRNA genes. Some of these tRNA genes contain introns while others do not. One of these genes appears to be novel. Its transcript can be folded into a tRNA-like structure, in which the "D-loop" contains 27 residues. It appears to code for tRNA^{Glu}_{CUC} and lacks the 3'-terminal CCA sequence of the mature tRNA. The sequence CCGGU occurs twice within the "D-loop"; the first four bases of which can pair with each other to form a stem. Two symmetrical cuts within or just outside these sequences (i.e., at any of the six pairs of positions) can produce a typical tRNA containing all of the invariant and semi-invariant residues at their respective positions and an 18-base "intron". The gene occurs as a single copy in the genome, as revealed by Southern hybridizations, using an "intron" and both the "5'- and 3'-exon" specific probes. The Northern hybridizations by the two "exon" specific probes indicated that the gene is transcribed. For some of the further studies we have subcloned this tRNA gene in pBluescribe vectors

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(Stratagene) in such a way that T7 RNA polymerase produces the transcripts which contain the complete sequence of the tRNA from position 2 through 71 (of the mature tRNA), and all of the "intron" and, in addition, some extra sequences at the 5' and 3' ends derived from the multiple cloning sites of the vectors.

In an effort to study the synthesis of the modified nucleosides of tRNAs, we are cloning the genes for archaebacterial tRNA-modifying enzymes. We have isolated a recombinant plasmid containing an approximately 9 Kb EcoRI genomic fragment of Sulfolobus in the pUC 19 vector. This fragment seems to contain genes for two tRNA-methyltransferases. The nucleoside composition of the tRNAs isolated from IPTG (isopropylthiogalactoside) induced E. coli cells containing this plasmid, as analysed by Liquid Chromatography/Mass Spectrometry (LC/MS), revealed the presence of N²-methylguanosine (m²G) and 1-methyladenosine (m¹A) in these tRNAs. These two modified nucleosides are known to be present in Sulfolobus, but are not normally present in E. coli tRNAs. It appears that at least some of the thermophilic archaebacterial genes are expressed when cloned in E. coli and that at least some of their enzymes are functional in the E. coli environment.

We have also cloned several tRNA genes of Thermococcus. The structures of these genes are being analyzed.

Plans for next year:

During the remaining period of this contract, all of the above mentioned works will be continued.

Initially, we shall determine whether the "intron" sequence (18 bases) in the above mentioned tRNA^{Glu} gene is actually spliced out or whether it exists as part of the mature tRNA. If it is spliced out, then we shall try to determine how its splicing is different from splicing other introns.

Initially the insert of clone carrying m²G and m¹A modification activities will be gradually deleted from the ends to determine the minimum size of the insert required to express these activities. These genes and their flanking regions will be sequenced. These genes will then be fused, in phase, to the lac Z gene of the pUC 18 or 19 vector with an aim of producing large quantities of the enzymes required for further studies.

In addition, we shall start sequencing mature tRNAs of Sulfolobus. Initially, Glu isoacceptor will be sequenced to determine whether the above mentioned tRNA has the extra 18 base in the mature tRNA.

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ONR Code 1141
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ONR Code 12
800 N. Quincy Street
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Chemical and Biological Sci Div
Army Research Office
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Technical Information Div, Code 2627
Washington, DC 20375

BELAS, M. Robert
Center of Marine Biotechnology
University of Maryland
600 East Lombard Street
Baltimore, MD 21202

BLAKE, III, Robert C.
Department of Biochemistry
Meharry Medical College
Nashville, TN 37208

BLAKEMORE, R. P.
Department of Microbiology
University of New Hampshire
Durham, New Hampshire 03824

BURCHARD, Robert P.
Department of Biological Sciences
Univ of Maryland-Baltimore County
Catonsville, MD 21228

CHAPMAN, David J.
Department of Biology
UCLA
405 Hilgard Avenue
Los Angeles, CA 90024

CLARK, Douglas S.
Dept of Chemical Engineering
University of California
Berkeley, CA 94720

COLWELL, Rita
Maryland Biotechnology Institute
University of Maryland
Microbiology Building
College Park, MD 20742

COOKSEY, Keith E.
Department of Microbiology
Montana State University
Bozeman, MT 59717

DANIELS, Charles J.
Department of Microbiology
Ohio State University
484 West 12th Avenue
Columbus, OH 43210

DANIELS, Lacy
Department of Microbiology
University of Iowa
College of Medicine
Iowa City, IA 52242

DENNIS, Patrick P.
Department of Biochemistry
University of British Columbia
2146 Health Sciences Mall
Vancouver, B.C. V6T 1W5

DOOLITTLE, W. Ford
Department of Biochemistry
Dalhousie University
Halifax, Nova Scotia
CANADA B3H 4H7

EISENBERG, Henryk
The Weizmann Institute of Science
Dept of Polymer Research
P.O. Box 26
Rehovot 76100, Israel

EPEL, Dvid
Hopkins Marine Station
Stanford University
Pacific Grove, CA 93950

FELBECK, Horst
Marine Biology Research Division
Scripps Institution of Oceanography
University of California - San Diego
La Jolla, CA 92093

FISHER, Charles R.
Marine Science Institute
University of California-Santa
Barbara
Santa Barbara, CA 93106

GIBOR, Aharon
Marine Science Institute
University of California
Santa Barbara, CA 93106

GONZALEZ, Elma
Department of Biology
UCLA
Los Angeles, CA 90024

GREENBERG, Everett P.
Department of Microbiology
University of Iowa
Iowa City, Iowa 52242

GUNSALUS, Robert P.
Department of Microbiology
UCLA
405 Hilgard Avenue
Los Angeles, CA 90024

GUPTA, Ramesh
Southern Illinois University
Dept of Chem and Biochemistry
Carbondale, IL 62901

HASTINGS, J. Woodland
Biological Laboratories
Harvard University
16 Divinity Avenue
Cambridge, MA 02138

HAYGOOD, Margo
Marine Biology Research Division
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093

JENSEN, Roy A.
Department of Microbiology
University of Florida
Gainesville, FL 32611

KELLY, Robert M.
Dept of Chemical Engineering
The Johns Hopkins University
Baltimore, MD 21218

KIRCHMAN, David L.
College of Marine Studies
University of Delaware
Robinson Hall
Newark, DE 19716

KONISKY, Jordan
Department of Microbiology
University of Illinois
809 Sout Wright Street
Champaign, IL 61820

LEADBETTER, Edward R.
Dept of Molecular and Cell Biology
University of Connecticut
Box U-131
Storrs, CT 06268

LIAO, Hans H.
Biotechnology Center
University of Wisconsin
1710 University Avenue
Madison, WI 53705

LIDSTROM, Mary E.
Keck Laboratories 138-78
California Institute of Technology
Pasadena, CA 91125

MITCHELL, Ralph
Division of Applied Sciences
Harvard University
125 Pierce Hall
Cambridge, MA 02138

MORSE, Daniel E.
Marine Science Institute
University of California
Santa Barbara, CA 93106

NADATHUR, Govind S.
Marine Science Institute
Univ Cal-Santa Barbara
Santabarbara, CA 93106

NEALSON, Kenneth H.
Center for Great Lakes Studies
University of Wisconsin-Milwaukee
600 E. Greenfield Avenue
Milwaukee, WI 53204

OLSEN, Gary J.
Indiana University
Department of Biology
Jordan Hall 138
Bloomington, Indiana 47405

PACE, Norman R.
Department of Biology
Indiana University
Bloomington, IN 47405

PREZELIN, Barbara B.
Marine Science Institute
University of California
Santa Barbara, CA 93106

REEVE, John N.
Department of Microbiology
Ohio State University
484 West 12th Avenue
Columbus, OH 43210-1292

ROSEMAN, Saul
Department of Biology
Johns Hopkins University
Baltimore, MD 21218

SEARCY, Dennis G.
Zoology Department
University of Massachusetts
Amherst, MA 01003

SILVERMAN, Michael
Agouron Institute
505 Coast Blvd. South
La Jolla, CA 92037

SMIT, John
Department of Microbiology
University of British Columbia
#300 - 6174 University Blvd
Vancouver, British Columbia
V6T 1W5 CANADA

SPUDICH, John L.
Dept of Anat and Structural Biolgy
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461

STAHL, David A.
College of Veterinary Medicine
University of Illinois
Urbana, IL 61801

SWIFT, Hewson
Dept of Molec Genetics
and Cell Biology
University of Chicago
1103 East 57th Street
Chicago, IL 60637

TAYLOR, Gordon T.
Hawaii Institute of Geophysics
University of Hawaii
2525 Correa Road
Honolulu, HI 96822

TOSTESON, Thomas R.
Department of Marine Sciences
University of Puerto Rico
Mayaguez, PR 00709

TRENCH, Robert K.
Marine Science Institue
University of California-Santa
Barbara
Santa Barbara, CA 93106

WALEH, Nahid
Molecular Biology Department
SRI International
333 Ravenswood Avenue
Menlo Park, CA 94025

WHITE, David
Institute of Applied Microbiology
University of Tennessee
P. O. Box X, Building 1503/6
Oak Ridge, TN 37831

WOESE, Carl R.
Genetics Department
University of Illinois
515 Morrill Hall
Urbana, IL 61801

YAYANOS, A. Aristides
Physiological Research Laboratory
Scripps Institution of Oceanography
University of California-San Diego
La Jolla, CA 92093

ZINDER, Stephen H.
Department of Microbiology
Cornell University
Stocking Hall
Ithaca, NY 14853